Appendix E

Evaluation of Biological Conditions Important to Monitored Natural Attenuation at the Building 51/64 Site

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Final Project Report
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Executive Summary

The Building 51/64 Site, located at Lawrence Berkeley National Laboratory (LBNL), is contaminated with chlorinated organic compounds including 1,1,1-trichloroethane (TCA), perchloroethene (PCE) and trichloroethene (TCE). TCA, PCE, and TCE were used for cleaning vacuum pumps and other equipment at the southeast corner of Building 64 area prior to 1980. Degradation products of these compounds; including 1,1-dichloroethane (1,1-DCA), 1,1-dichloroethene (1,1-DCE), and cis-1,2-dichloroethene (cis-DCE); have also been detected in the groundwater at the site. In 2000, the highly contaminated sediment identified as the source area was excavated as an interim corrective action.

There is an accumulating base of information indicating that natural attenuation of chlorinated compounds is occurring at the Building 51/64 Site. Data collected since the source was excavated indicates that concentrations are significantly decreasing in response to the removal action. The absence of cis-DCE in the source area and the presence of cis-DCE in the down-gradient extent of the plume is strong evidence natural biodegradation is occurring. Conrad (2000) used stable isotope measurements to prove that biological degradation of contaminants was occurring naturally at the site. Shifts in the stable carbon isotope ratios of TCE, cis-DCE, and vinyl chloride along the length of the plume flow path showed anaerobic biodegradation was occurring at the site. The stable isotope analysis also demonstrated that vinyl chloride was being completely converted to ethane (Conrad, 2000).

In 2002, a review of the Building 51/64 Site was conducted by the US Department of Energy (DOE) Subsurface Contaminant Focus Area (SCFA) technical assistance team, consisting of DOE and non-DOE experts in environmental remediation. The SCFA team evaluated plume hydrology, site geology, monitoring data, and other available information. The SCFA team's conclusion was that Monitored Natural Attenuation (MNA) was the most appropriate technology for remediation of the Building 51/64 Site (SCFA 2002). MNA was recommended because the source area has been successfully removed, dispersion of the plume is limited, transport of the plume is slow, and migration is confined by complex geology and formational constraints. The extremely low hydraulic conductivity has restricted the plume expansion to tens of meters over several decades. Ground water flows primarily within surficial units (artificial fill and colluvium) and in the sedimentary rocks of the low permeability Orinda formation. All of the contaminants are inherently biodegradable and daughter compounds (products of biodegradation) found at the site indicated that natural attenuation had already occurred. The report suggested that studies be conducted to further evaluate the feasibility of applying MNA at this site (SCFA 2002).

The objective of this study was to determine if the environmental conditions at the Building 51/64 Site were appropriate for supporting the natural or intrinsic bacterial degradation of chlorinated compounds. Appropriate environmental conditions are requisite for the application of MNA for site remediation. Historical data was evaluated and sediment and water samples were collected and analyzed. Analysis of the terminal electron acceptors (TEA) necessary for aerobic and anaerobic biological activity indicated five distinct zones along the hydraulic gradient of the plume. Oxygen, nitrate, sulfate, iron, manganese, and methane concentrations in the ground water demonstrated that significant biological activity was occurring and that the redox conditions in up-gradient area from the plume were oxidizing, the old source area was mixed oxidizing/reducing, and that the plume gradually became more and more reducing along

its length. The gradually increasing reducing nature of the plume also suggested adequate electron donor was present to sustain natural biological activity.

The gradient of daughter products found in the ground water in the different zones also suggests that both aerobic and anaerobic biodegradation is occurring in the hydraulic gradient of the plume. 1,1,1-TCA is completely converted to 1,1-DCA by the time it reaches the middle part of the plume. PCE, and TCE decline and their daughter products cis-DCE and vinyl chloride (VC) increase and then decline along the natural gradient of the plume. Sediment pH and moisture were well within the limits of normal activity for chlorinated solvent degrading organisms. Aerobic and anaerobic culturable counts for sediment samples were from 10^3 to 10^6 colony-forming-units (CFU) per gram sediment. These culturable densities are considered normal for an un-stimulated site and suggest normal microbial activity and a healthy soil.

Chlorinated solvent degrading bacteria were detected in sediments and groundwater collected from the site. These included *Dehalococcoides* species, which degrade chlorinated solvents anaerobically, and toluene-degrading bacteria, which degrade chlorinated solvents aerobically. Sediment samples were also used in treatability tests for TCE and VC. These tests demonstrated that VC is quickly mineralized to carbon dioxide, but that TCE degradation takes a significantly longer time. Total organic carbon analysis indicated that 2-5% of total dry weight of the sediment is organic carbon, and a significant fraction of this is soluble and therefore bioavailable. Biological oxygen demand studies of the sediment also demonstrated that the sediment carbon was bioavailable and therefore could support the biodegradation of the chlorinated solvents.

The lines of evidence for this and previous studies that demonstrate that monitored natural attenuation is feasible for the remediation of the Building 51/64 Site are as follows:

1. The contaminant source has been identified and removed.
2. Dispersion of the plume is limited, transport of the plume is slow and migration is confined due to low hydraulic conductivity, complex geology and formational constraints.
3. All contaminants present are inherently biodegradable under both aerobic and anaerobic conditions.
4. Bacteria capable of degrading chlorinated solvents were isolated from the site, including *Dehalococcoides* species, which are unique bacteria in that they are able to completely mineralize chlorinated solvents under anaerobic conditions.
5. Biodegradation daughter products are present and increase as the plume moves away from the source area.
6. The concentration of parent and daughter products along the plume hydraulic gradient also showed biodegradation was occurring.
7. Isotopic analysis of the contaminants and daughter products indicate that they are being biodegraded, and that VC is being completely converted to ethane.
8. The plume can be divided into five distinct zones that have different degrees of biological activity, typical of a contaminant plume with natural biodegradation.
9. Terminal electron acceptors in the gradient of the plume cross-validated the type of biological activity that would be present in terms of aerobic/anaerobic and the reducing nature of the zone.

10. Sediment pH, moisture, and organic carbon content are sufficient to support natural biodegradation.
11. Culturable bacteria densities indicated that microbial activity was normal and high enough to support significant biodegradation activity.
12. Treatability tests with VC demonstrated complete mineralization using sediment from the site. Rates of VC biodegradation were high compared to other contaminated sites.
13. Organic carbon analysis and bioavailable carbon measurements also demonstrated that the site has enough secondary carbon and that it is bioavailable to support natural biodegradation of chlorinated solvents.

The results of this study further support the application of MNA as the best remediation option for the Building 51/64 Site.

Introduction

The Building 51/64 Site

A plume of volatile organic compound (VOC) contaminated groundwater, known as the Building 51/64 VOC plume, extends from the southeast corner of Building 64, under Buildings 64 and 51B (Figure 1). This plume is defined by the presence of chlorinated ethanes such as 1,1,1-trichloethane (TCA) and its degradative daughter, 1,1-dichloroethane (DCA). This plume also contains lower concentrations of other solvents such as the chlorinated ethenes – PCE, TCE and 1,1-DCE. In calendar year 2000, prior to a source removal (excavation) effort, chlorinated solvents were detected at high concentrations (greater than 100,000 µg/L) in the most concentrated portion of the Building 51/64 VOC plume. In this area of the plume, near the original source, contaminant solvents were comprised primarily of 1,1,1-TCA (82%) and 1,1-DCA (7%). The contaminant profile shifted toward the less chlorinated (i.e., more weathered) solvents and overall concentrations decreased as distance from the source increased. This pattern, combined with preliminary stable isotope data discussed below, suggests that some natural degradation of the solvents is occurring in the plume as it migrates. In 2000, highly contaminated sediment was excavated from the source area as an interim corrective measure. According to the LBNL staff, recent data indicate that concentrations are significantly decreasing in response to the removal action. Figure 1 shows the original (circa 2000) extent of VOCs in groundwater in the Building 51/64 area.

The source area has been successfully removed, dispersion of the plume is limited, transport of the plume is slow, and migration is confined by complex geology and formational constraints. The extremely low hydraulic conductivity has restricted the plume expansion to tens of meters over several decades. Groundwater flows primarily within the surficial units (artificial fill and colluvium) and in the sedimentary rocks of the low permeability Orinda formation. All of the contaminants are inherently biodegradable and many of the compounds in the groundwater are daughter products of biodegradation that has already occurred.

Natural Attenuation

The chlorinated compounds found at 51/64 are biodegradable and can be transformed by a number of different bacteria under a variety of environmental conditions; however, both aerobic and anaerobic biodegradation processes require the presence of secondary organic carbon sources, such as natural organic carbon or methane, to drive forward chlorinated compound degradation (Bagley and Gosset 1990; Bradley and Chapelle 1996 and 1997; Bradley et al. 1998; Cabirol et al. 1998; Chang and Alvarez-Cohen 1995; DeBruin et al. 1992; DiStefano et al. 1991; Holliger et al. 1992). Anaerobic reductive dechlorination of chlorinated ethenes also occurs at low redox conditions and therefore can be inhibited by competing terminal electron acceptors (TEA), thus TEA can indicate the probability of reductive dechlorination at a site (Figure 2). If extensive biological degradation of chlorinated compounds is occurring at a contaminated site, natural attenuation of the contaminants will occur. If, in addition, the hydrologic and geologic conditions are appropriate, it is possible to apply “monitored natural attenuation” (MNA) as a remediation strategy for contaminated areas (Wiedemeier et al. 1996).

A previous study developed convincing evidence that natural attenuation, primarily biological degradation, was occurring at the Building 51/64 Site (Conrad, 2000). Evidence that natural attenuation was occurring at the site included the absence of cis-DCE in the source area and the presence of cis-DCE in the down gradient extent of the plume. Shifts in the stable

carbon isotope ratios of TCE, cis-DCE, and vinyl chloride along the length of the plume flow path indicated that anaerobic reductive dechlorination was occurring spontaneously at the site (Conrad 2000). Reductive dechlorination of TCE, PCE, and other chlorinated compounds is a well-characterized biodegradation process and is the primary degradative process relied upon during the application of MNA for site remediation (Wiedemeier et al. 1996, Hendrickson et al. 2002). Carbon isotope measurements were used to demonstrate that vinyl chloride, the terminal chlorinated product of the reductive dechlorination degradation pathway, was being converted completely to ethane in the Building 51/64 plume, even under anaerobic conditions (Conrad 2000). This is an especially important finding, as the accumulation of vinyl chloride under anaerobic conditions is a negative indicator for the application of MNA for site remediation (Wiedemeier et al. 1996, Hendrickson et al. 2002).

In 2002, a review of the Building 51/64 Site was conducted by the DOE SCFA technical assistance team, consisting of DOE and non-DOE experts in environmental remediation. The SCFA team evaluated plume hydrology, site geology, monitoring data, and other available information and came to the conclusion that MNA was the most appropriate remediation technology for application at the Building 51/64 Site (SCFA 2000). The report suggested that studies be conducted to further evaluate the feasibility of applying MNA at this site (SCFA 2002).

The objective of this study was to determine if the environmental conditions at the Building 51/64 Site were appropriate for supporting the bacterial degradation of chlorinated compounds. Historical data was evaluated and sediment and water samples were collected and analyzed. Lines of evidence were developed demonstrating that conditions at the site are very favorable for microbial activity and chlorinated solvent biodegradation.

Methods

Sample collection and preparation

Sediment core samples were collected from the Building 51/64 Site on January 13, 2003 using a push core sampler. Water samples from adjacent wells were collected with a Teflon bailer on January 14 and 15. Sediment samples were collected separately from depths that were saturated year round (saturated zone) and from depths that were saturated intermittently as a function of seasonal and annual changes in groundwater depth (seasonal zone). Sample depth intervals, sediment core identification numbers, and identification of associated wells are listed in Tables 3-5.

Sediment samples were collected in four-foot long polycarbonate tubes using sterile techniques. Sediment cores were cut into 2' lengths and were brought to the Biokinetics Laboratory at LBNL from the drilling site in a chilled cooler under a CO_2 atmosphere and placed immediately in a 4°C refrigerator. Intact sediment cores were provided to researchers at the University of California Berkeley (UC Berkeley) for microcosm studies. For experiments and analysis conducted at LBNL, cores were processed under an argon atmosphere in a glove bag using sterile technique. Approximately 5 cm of sediment from each end of the cores were removed and discarded. Each individual core sample was removed from the collection sleeve and thoroughly mixed before being transferred to Whirlpak bags and stored at 4°C until analyzed.

Carbon, moisture and pH analysis

Moisture and organic carbon content of the sediment samples were determined following Standard Methods 2540G for the determination of total, fixed, and volatile solids in solid and semisolid samples (APHA 1998). Sediment organic matter content was measured in duplicate samples from each sample interval using gravimetric measurement before and after combustion. Combusted samples were wetted and dried before measurement to compensate for volatile loss of carbonates.

Sediment pH was determined by mixing a one to one slurry of sediment and distilled deionized water (weight to weight). The slurry was equilibrated and the pH was measured in the aqueous phase after centrifugation.

Bioavailable sediment organic carbon was measured using a biokinetic assay adapted from Standard Method 5210 for the determination Biochemical Oxygen Demand in water (APHA 1998). Sediment core samples were mixed under an aerobic atmosphere to pre-oxidize reduced metals and other chemically reactive species before sub-samples were taken to use in the assay. Cores from saturated and seasonal zones were analyzed separately. Approximately eight grams of sediment (dry weight) was added to 300 mL of BOD buffer in a standard BOD bottle. Oxygen concentration was measured in triplicate samples at the initiation of the assay and at appropriate intervals (approximately every five days) over the course of the assay. BOD Buffer solution was prepared by adding 1 mL each of four stock solutions per liter of Millipore de-ionized water. Solution one contains (0.025 g $FeCl_3 \bullet 6H_2O$/liter) solution two contains (8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \bullet 7H_2O$, and 1.7 g NH_4Cl/liter), solution three contains (22.5 g $MgSO_4 \bullet 7H_2O$/liter), and solution four contains (27.5 g $CaCl_2$/liter). Initial oxygen concentrations were determined on an YSI Model 5000 oxygen meter with self-stirring probe that had been calibrated using the Winkler titration method. BOD bottles were sealed and

placed in a dark incubator at 20°C. Dissolved oxygen readings were reported as micrograms of dissolved oxygen consumed per gram dry weight of sediment per day.

Soluble total organic carbon (TOC) was measured in ground water samples collected from the wells adjacent to sediment core collection points. TOC was determined on acid preserved samples using a Tekmar Apollo 9000HS combustion/infrared analyzer according to Standard Method 5310A (APHA 1998).

Biological analysis

Aerobic heterotrophic bacteria were enumerated using plate counts on R2A agar. Starting from approximately 1 gram of sediment sample, serial dilutions were made in a buffer solution containing 8.4 g NaCl, 0.3 g KH_2PO_4, 0.6 g Na_2HPO_4, 0.1 g $MgSO_4$, 0.1 g peptone, in 1000 mL de-ionized water. One hundred microliters of each dilution sample were plated in triplicate and the colonies that formed counted 7 and 14 days later.

Toluene degrading bacteria were enumerated using mineral media plates incubated in a toluene atmosphere. Toluene served as the sole carbon and energy source in an aerobic atmosphere.

Anaerobic heterotrophic plate counts were conducted by CytoCulture International, Inc. (Richmond, CA). Sterile agar plates (100 x 15 mm) were prepared with minimal salts medium and 2.35% heterotrophic plate count agar at pH 6.8 without any other carbon sources or nutrients added. Plates were setup and poured in a Coy anaerobic glove box under strict anaerobic conditions (atmosphere of nitrogen, carbon dioxide and hydrogen). Plates were inoculated with 1.0 mL of a 0.2% sodium pyrophosphate extract of the sample, or log dilutions of the extract, in triplicate at sample dilutions of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. The heterotrophic plates were counted after 21-28 days incubation in the glove box at ambient temperature.

The presence of *Dehalococcoides ethenogenes* was investigated using molecular techniques. Anaerobic bacteria from sediment and ground water were enriched in the presence of chlorinated solvents and tested for the presence of *D. ethenogenes* by polymerase chain reaction (PCR) assay. DNA was extracted from the anaerobic enrichment by a bead beating procedure using the MoBio Ultra Clean Soil DNA Kit and protocol. PCR primers were designed to target a 45 basepair fragment of the 16s ribosomal DNA exclusive to *D. ethenogenes*. The target DNA fragment was amplified by PCR, and the 45 basepair PCR product was detected.

The 16s rDNA amplification was performed using an Eppendorf Mastercycler Gradient Thermocycler. The 30 µl reactions contained 3 µl of 10x PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each dioxynucleoside triphosphate, 300 nM of each forward and reverse primers, 0.15 µl AmpliTaq Gold, and 1 µl of DNA. The following thermocycling program was used: 12 minutes at 94°C for denaturation, followed by 30 cycles of 1 minute at 94°C, 45 seconds at 50°C, and 2 minutes at 72°C. A final extension step of 12 minutes at 72°C followed by cooling at 4°C was performed. The PCR product was visualized using an Agilent 2100 Bioanalyzer and the accompanying DNA 500 reagents and chip. This PCR method was run concurrently with positive and negative controls.

Microcosms for the detection of TCE degrading bacteria were prepared at LBNL using sediment samples from both saturated and unsaturated depths. All microcosms were prepared as triplicates in a glove bag under an Argon atmosphere. Each microcosm consisted of a 40 mL VOA vial sealed with Mininert cap, contained 20 mL of liquid and approximately 20 g sediment sample from refrigerated Whirlpak bags. All experimental and control microcosms contained 10 mL of Sole Source Carbon (SSC) medium or SSC-Lactate medium and 500 µL trichloroethylene

(TCE) solution. Killed controls also contained 3 mL of a 1 g/L solution of mercuric chloride. The balance of liquid in each vial was brought to 20 mL with sterile distilled-deionized water. SSC media was prepared with 1.00 g KH_2PO_4, 0.86 g Na_2HPO_4, 1.00 g NH_4Cl, 0.12 g $MgSO_4 \cdot 7H_2O$, 0.06 g $CaCl_2 \cdot 2H_2O$ to make 1 liter. SSC-Lactate media was prepared as SSC with the addition of 2 mL 60% sodium lactate syrup. Both media had 1 mL of the redox indicator, resazurin (1g/L solution) added before being boiled and then autoclaved and tightly capped to exclude as much oxygen as possible. Following setup, the microcosms were placed in a 25°C incubator on a shaker at 110 rpm. Initially, TCE measurements were made at day one followed by once per week for the next 4 weeks, then about once every 2 weeks thereafter.

TCE concentrations were determined using a Hewlett Packard 6890 gas chromatograph with a micro electron capture detector. Fifty µL headspace injections were made onto a (J&W scientific) 30 m x 250 um DB5ms capillary column using a split ratio of 40 to 1. Injector temperature was constant at 240°C while the column was held at 40°C for 3 minutes then increased to 160°C at 15°C /minute for a total run of 11 minutes. Calibration curves were obtained from headspace analysis of aqueous dilutions prepared by mixing the pure solvent until dissolved in water for several hours in a sealed container with minimal headspace.

Microcosms for the detection of vinyl chloride degrading bacteria were prepared and tested at UC Berkeley. For microcosm preparation, sediment cores were bulked together in an anaerobic chamber, and the sediment was manually homogenized using a sterile spatula to break sediment clumps and remove rocks. Homogenization also released the indigenous volatile contaminants from the sediment. Groundwater used in construction of the aerobic microcosms was mixed on a stir plate open to the air, while nitrogen was bubbled through the groundwater for anaerobic microcosms to allow contaminants to volatilize. Groundwater samples were collected from monitoring wells adjacent to each soil boring (Table 4). Monitoring wells were purged and allowed to recharge overnight before groundwater samples were collected from a depth of approximately 30 feet below ground surface (bgs). All serum bottles and stoppers used for the study were autoclaved. Each experimental microcosm consisted of a 68 mL Wheaton glass serum bottle containing 15 grams of sediment, 15 mL of groundwater, about 38 mL of headspace, 250 µL of vinyl chloride, and possibly nutrients and/or co-substrates. Nitrogen and phosphorous was added to selected bottles as 5 mM diammonium phosphate to test the effect of the presence of nutrients on vinyl chloride degradation. Benzene and toluene were added at 35 mg/L each to selected aerobic bottles to examine possible inhibition or enhancement of vinyl chloride degradation by BTEX compounds. Anaerobic microcosms were amended with toluene alone or lactate as 0.02 M lactic acid to provide electron donors for anaerobic degradation. Killed and abiotic controls were constructed as previously described. The bottles were sealed using butyl rubber stoppers and secured with aluminum crimps purchased from Bellco Glass Co. Sediment was incubated at room temperature in the dark for one week before the construction of the anaerobic microcosms and for one month before construction of aerobic microcosms. After the experiments began, anaerobic microcosms were stored statically in an anaerobic chamber, and aerobic microcosms were stored on a shaker table, both at room temperature in the dark. Anaerobic microcosms were monitored for 126 days, and aerobic microcosms were monitored for 89 days.

In the UC Berkeley microcosm studies, gas phase components were monitored over time by headspace analysis using gas chromatography. Vinyl chloride disappearance, ethene production, and methane production in microcosms were monitored by withdrawing 30 µL of gas from the headspace with a gas-tight syringe and injecting it into a Hewlett Packard 5890

Series II gas chromatograph (GC) equipped with a flame ionization detector. The production of carbon dioxide and consumption of oxygen were monitored using 0.2 mL of gas from the headspace into a Hewlett Packard 5890 Series II GC with a thermal conductivity detector. Samples were collected at time intervals appropriated by degradation progress, with a week maximum interval. Between every injection, the syringes were flushed three times with acetone and suction-dried to avoid cross-contamination. Standard calibration curves (including 4 data points and with R^2 values greater than 0.98) were generated daily to convert GC data to accurate concentration measurements.

Microbial biomass was measured using a Coomassie Protein Assay Reagent Kit from Pierce Biotechnology. One gram of saturated sediment from microcosms sacrificed at the start of the experiments and stored at 4°C throughout the experiments and from microcosms at the end of the experiments was processed with calibration standards according to a modified test tube protocol. Protocol adjustments include the addition of 200 mM final concentration sodium hydroxide to the samples, three cycles of freezing and thawing the samples, and 20 minutes of boiling to aid in cell lysis, followed by a 15 minute centrifugation at approximately 12,000 times gravity to separate protein from cell debris. An IEC Micromax RF centrifuge was used. One hundred µL of the supernatant was then mixed with 500 µL of the Coomassie reagent and incubated at room temperature for 10 minutes. The absorbance of the sample was measured on a Perkin Elmer Lambda 14 UV/Vis spectrometer at 595 nm. Absorbance was converted to protein concentration using the standard calibration curve. Protein calibration standards were prepared identically to microcosm samples. The change in protein concentration in the samples from the beginning to the end of the experiments was compared with the expected yield considering the amount of vinyl chloride consumed throughout the experiments.

Results and Discussion

Analysis of Historical Data

Historical data from the Building 51/64 Site were compiled and analyzed to determine if the historical record indicated a pattern of biological activity consistent with the application of MNA for treatment of the 51/64 plume. Data included in this analysis were measurements for terminal electron acceptors (TEA) conducted in December of 1997 and methane concentration measurements from October 2002. Additionally, chlorinated solvent and chlorinated solvent degradation products data from fiscal year 1999 were also evaluated.

Analysis of TEA and methane data

Historical data was interpreted in the context of the site hydrology and it was determined that the 51/64 plume could be divided into five biological zones (Figure 3). Zone 1 represents the up-gradient, background ground water that is not influenced by the contamination that occurred at the Southeast corner of Building 64. Two wells in Zone 1 (MW 90-4 and MW 90-5, indicated in Figure 3 by ⊗) were found to be influenced by an up-gradient source of contamination (Building 71B plume) and data from these wells was not included in this analysis. Water from SB 64-99-7 is apparently free of contamination and may represent clean groundwater moving into the site from the North-northwest. Data from this well was not included in this study, but this well could serve as an additional background collection point in future studies, particularly if MW 90-6 is compromised. Zone 1 historical data (Table 1) indicate that the up-gradient groundwater entering the site contains oxygen, nitrate and sulfate, electron acceptors used in both the aerobic and anaerobic degradation of chlorinated solvents (Bagley and Gosset 1990; Bradley and Chapelle 1996 and 1997; DiStefano et al. 1991; Wiedemeier et al. 1996, see Figure 2). The TEA profile does not indicate the presence of significant ground water associated biological activity in this zone.

Zone 2 corresponds to the source area. Source removal was accomplished by excavation and an approximately 20 square foot area surrounding MW 51-96-18 was removed and backfilled with gravel (SCFA 2002). TEA data from this area (Table 1) show that dissolved oxygen (DO) is lower than in Zone 1, but that nitrate and sulfate are not significantly lower than background. The TEA profile in this zone indicates the dominance of oxidized conditions in this area. The predominant biological activity in this zone would be aerobic metabolism, but the rate of activity is sufficiently low that the rate of oxygen utilization does not exceed the rate of reoxygenation from diffusion and the in-flow of oxygenated groundwater from Zone 1. Zone 3 and Zone 4 are distinct biological zones as indicated by historical TEA and methane data (Table 1). Wells in Zone 3 still had measurable oxygen concentrations, but increased concentrations of ferrous iron and divalent manganese indicate the occurrence of areas of reduced or anoxic conditions in this zone (Table 1). The presence of soluble iron and manganese is a more reliable indicator of low redox conditions in the subsurface than the measurement of oxygen concentrations in the well water. The presence of anoxic or anaerobic areas in Zone 3 is also indicated by reduced nitrate and sulfate concentrations (compared to Zone 1 and 2) and the presence of low concentrations of methane. Zone 3 TEA conditions indicate that both aerobic and anaerobic chlorinated solvent degrading bacteria could be active in this area. The co-occurrence of both oxygen and methane in proximity suggest that aerobic, cometabolic

biodegradation of chlorinated organic compounds by methane-oxidizing bacteria could be an important natural attenuation process in this zone.

Zone 4 appears to be fully anaerobic. Only trace concentrations of oxygen are found in any well, soluble iron and manganese concentrations are high, nitrate and sulfate are reduced, and methane concentrations are high (Table 1). The anaerobic conditions found in Zone 4 also suggest that this is a biologically active zone. Conditions in Zone 4 are appropriate for the growth of strict anaerobes, as evidenced by the high concentrations of methane. The dominant natural attenuation process in this area would be reductive dechlorination, which requires strict anaerobic conditions.

Zone 5 is down-gradient from Zone 4 and has a similar TEA profile as Zone 4, but does not have any detectable solvent contamination. The anaerobic conditions in Zone 5 suggests that groundwater from Zone 4 may be entering Zone 5, since the up-gradient, reference wells indicate that uncontaminated ground water in the area is typically aerobic (Zone 1, Table 1). Since the extent of chlorinated solvent contamination is limited to Zones 2, 3 and 4 (Table 2) and the chemical similarity between Zones 4 and 5 suggest that contaminated groundwater has migrated from Zone 4 to Zone 5, it is possible that groundwater in Zone 5 was once contaminated and has now been completely cleansed of chlorinated solvents by natural attenuation. The alternative interpretation of the TEA data is that the source of groundwater in Zone 5 is not from the Building 51/64 plume and Zone 5 is anaerobic for other reasons (e. g., up-gradient wells in Zone 1 are in natural bedrock, whereas Zone 5 wells are in fill material). To resolve this question, more information is needed on the hydrologic connection between Zones 4 and 5, the ground water travel time between the two zones, and the TEA profile of uncontaminated areas in substrata comparable with Zone 5. The detection of ethane or ethene, chlorinated solvent degradation products, in Zone 5 would also be further proof of natural attenuation is removing solvents between Zones 4 and 5. A better understanding of the link between Zone 4 and Zone 5 is needed before it can be demonstrated that natural attenuation has already resulted in the complete remediation of contaminated groundwater down-gradient of the Building 51/64 Site.

Analysis of chlorinated solvent and degradation product data

Chlorinated organic compound data from 1999 were examined to determine if there was historical evidence for the occurrence of natural attenuation at the Building 51/64 Site. Three chlorinated solvents, 1,1,1-trichloroethane (TCA), perchloroethene (PCE), and trichloroethene (TCE), were used for degreasing vacuum pumps at the Southeast corner of Building 64. TCE and TCA can be degraded under aerobic conditions by bacteria able to grow on methane, toluene, and other compounds via a process typically referred to as co-metabolism (Chang and Alvarez-Cohen, 1995). Aerobic TCE, and TCA degradation can result in complete mineralization, yielding CO_2 and chloride as final products (Aziz et al., 1999; Chang and Alvarez-Cohen, 1995). PCE can also be biodegraded in nominally aerobic environments, but studies have shown this is not actually aerobic, co-metabolic biodegradation, but rather anaerobic reductive dechlorination of PCE that takes place in localized, diffusion limited areas of the sediment (Enzien et al., 1994).

Anaerobic metabolism of chlorinated solvents occurs via a process termed reductive dechlorination, which can yield a number of intermediate chlorinated products before complete mineralization. Chlorinated intermediates products for PCE and TCE are cis-1,2-dichloroethene (cis-1,2-DCE), 1,1-DCE, trans-1,2-DCE, and vinyl chloride (Wiedemeier et al., 1996). Anaerobic biodegradation of TCA can produce the intermediate products 1,1-dichloroethane

(1,1-DCA) and monochloroethane (Wiedemeier et al., 1996). The presence of the chlorinated intermediates in well water samples indicates that natural biodegradation is occurring at the Building 51/64 Site.

Zone 1 wells were free of contamination. Zone 2 has measurable concentrations of the three parent compounds TCE, PCE, and 1,1,1-TCA as well as the degradation products 1,1-DCA, 1,1-DCE, and cis-1,2-DCE. The presence of biological degradation intermediates indicates that biological degradation, most likely anaerobic reductive dehalogenation, is occurring naturally in this zone. Vinyl chloride is not present, as might be expected, considering Zone 2 has a mixed aerobic and anaerobic TEA profile and vinyl chloride is subject to rapid biodegradation under aerobic conditions (West et al. 2003; Wiedemeier et al. 1996).

Zones 3 and 4 do not have measurable amounts of 1,1,1-TCA, but there are trace concentrations of the product 1,1-DCA. 1,1-DCA concentration declines slightly between Zones 3 and 4. Since the hydraulic connectivity between Zones 2, 3, and 4 is established, and the 1,1-DCA would not occur in groundwater that did not have TCA contamination at some time, this data can be interpreted with confidence as indicating that the parent compound 1,1,1-TCA has been removed completely by natural attenuation by the time groundwater has passed from Zone 2 to Zone 4.

The presence of 1,1-DCE, cis-1,2-DCE, and vinyl chloride are definitive evidence that natural biodegradation is occurring in Zones 3 and 4. The significance of the decline in TCE, PCE, and cis-1,2-DCE between Zones 3 and 4 cannot be interpreted using this data set. However the trend toward lower concentrations between Zones 3, 4, and 5 are consistent with the occurrence of natural attenuation at this site and a declining trend is consistent with criteria for determining the applicability of monitored natural attenuation for site remediation.

In summary, the analysis of historical TEA, methane, and chlorinated compound data are supportive of the use of MNA at the Building 51/64 Site. These data indicated that Zones 3 and 4 as the most biologically active areas of the site, and based on this analysis, sediments cores were collected in Zones 3 and 4 for further biological characterization.

Analysis of Sediment Cores and Groundwater Samples

Collection and preparation of sediment cores

Based on the analysis described in the previous section, it was determined that Zones 3 and 4 were the most biologically active. Sediment cores were collected from borings adjacent to MW 51-96-16 in Zone 3 and wells MW 51-97-12, MW 51-97-13, and MW 56-98-2 in Zone 4 for biological characterization (Figure 3).

Sediment cores were sectioned and categorized as either “seasonal” or “saturated.” Seasonal sediments were from the zone of the aquifer that is subject to variable saturation conditions as the water table rises and falls during the year. The saturated sediments were from zones of the aquifer that was below the lowest water table level recorded for the well adjacent to the soil coring. Seasonal and saturated zones were determined from long-term well monitoring data summarized in Table 3.

Analysis of biological conditions

Biological conditions at the Building 51/64 Site were evaluated to determine if they are appropriate for MNA. Sediment pH ranged from 7.5 to 8.4, well within the range compatible

with microbial activity. The sediment moisture content (between 14% and 22% by weight) is sufficient to allow bacteria growth in both the seasonal and saturated zones. Both the seasonal and saturated zone had the same moisture content during this sampling event because it took place in the rainy season.

Total heterotrophic bacterial populations at the site were measured using standard aerobic and anaerobic enumeration techniques. Culturable aerobic populations in both the saturated and unsaturated zones were in the hundred of thousands to millions of culturable bacteria per gram of sediment (Table 4). This density of culturable population is well within the range found for uncontaminated clay subsurface sediments and indicates that the bacterial community is not inhibited by unrecognized factors such as heavy metals or tannins in the sediment matrix.

Bacteria able to grow on a heterotrophic media under anaerobic conditions numbered in the tens of thousands in the sediment samples collected from the saturated depths (Table 4). As with the aerobic counts, the presence of easily culturable anaerobic populations indicates that the sediments are active and healthy. The ratio between the bacteria enumerations using aerobic and anaerobic plate counts (approximately 10 to 1) are also typical of healthy natural sediments. Direct bacterial counts, using activity indicators, could not be conducted on these sediments because of their high clay content and natural background fluorescence.

Analysis of chlorinated compound degradation potential

The isolation or detection of bacteria able to transform chlorinated compounds demonstrates biological degradation potential and is a further line of evidence often developed in support of MNA (Wiedemeier et al. 1996, Hendrickson et al. 2002). Direct enumeration techniques and microcosm studies were used to determine if bacteria able to transform chlorinated compounds were present at the Building 51/64 Site. Additionally, DNA was extracted from groundwater and sediment enrichment cultures and gene probes were used to demonstrate the presence of *Dehalococcoides ethenogenes*, a key bacteria in the anaerobic degradation of chlorinated solvents (Hendrickson et al. 2002).

Toluene degrading bacteria were detected by enumeration on bacterial media containing toluene as the sole carbon and energy source. Toluene degrading bacteria were present in all samples tested (Table 4). Bacteria able to grow on toluene contain toluene-oxygenase, which is an effective enzyme for the degradation of many chlorinated compounds including TCE (Chang and Alvarez-Cohen 1995). The activity of the toluene-oxygenase against TCE and other compounds will depend on the level of expression and the presence of oxygen. Many compounds, including natural organic matter, can induce expression of toluene degrading enzymes. The sediments contain significant concentrations of natural organic matter (Table 5). Zone 2 and 3 have oxygenated conditions and therefore this potential transformation mechanism is most significant for those zones.

Molecular analysis of sediment and groundwater from the site demonstrated that the site contained a subsurface population of the bacterium *Dehalococcoides ethenogenes*. *D. ethenogenes* is the only known organism capable of completely dechlorinating PCE and its daughter products to ethene (Maymó-Gatell et al. 1997, Hendrickson et al. 2002). In a wide-ranging study, it was demonstrated that the presence of *Dehalococcoides* in the contaminated subsurface environment was indicative that complete mineralization of chlorinated solvents was occurring and that undesirable intermediates would not accumulate at a site (Hendrickson et al. 2002). Detecting the DNA of *Dehalococcoides* in sediment and groundwater samples is definitive evidence that the indigenous microbial community at a contaminated site is able to

reductively dechlorinate chlorinated-ethenes (Hendrickson et al. 2002). Identifying this organism at the Building 51/64 Site is an important step in the application of MNA for site remediation (Wiedemeier et al. 1996, Hendrickson et al. 2002).

The presence of chlorinated solvent degrading bacterial populations were also measured using microcosm studies. In these studies, sediments were incubated under a variety of conditions in the presence of the target contaminant, either vinyl chloride or TCE. Microcosm tests for the presence of TCE degrading bacteria were conducted at LBNL; those for vinyl chloride were tested at UC Berkeley. Activity against the contaminant was indicated by measuring the removal of the target compound over time.

In the UC Berkeley studies, degradation activity against vinyl chloride was observed under both aerobic and anaerobic conditions (Figure 4). Ethene was produced stochiometrically from vinyl chloride in anaerobic conditions, indicating the vinyl chloride was completely transformed to a non-toxic product (Figure 5). This result is particularly significant, because the accumulation of vinyl chloride under (anaerobic) conditions favorable for the reductive degradation of TCE and other chlorinated solvents is undesirable at a site relying on MNA for remediation (Wiedemeier et al. 1996). This result also confirms the result of Conrad (2000), who used stable isotope techniques to show that the biological reduction of vinyl chloride to ethene was occurring in-situ at the Building 51/64 Site. The presence of bacteria able to anaerobically degrade vinyl chloride is supportive of MNA for the Building 51/64 Site.

The studies on vinyl chloride degradation conducted at UC Berkeley were part of a larger study comparing vinyl chloride degradation under different conditions and between different sites (West et al. 2003). The vinyl chloride degrading activity observed using sediments from the Building 51/64 Site was greater than that of sediments from other sites tested in the same study. The addition of nutrients, such as nitrogen or phosphorous, or electron donors, such as lactate or toluene, was not necessary for vinyl chloride degradation to occur (West et al. 2003). This further indicates that active bioremediation strategies, where supplemental materials such as nitrogen, phosphorous or organic carbon are introduced into the groundwater, are not necessary and that natural attenuation is appropriate for the Building 51/64 Site.

At LBNL, microcosms were constructed to measure the presence of bacteria able to reductively dechlorinate TCE. Microcosms were constructed with and without the addition of a supplemental electron donor (lactate). In contrast to the results for vinyl chloride, microcosms incubated for up to 184 days did not demonstrate significant TCE removal (data not shown). The field data clearly demonstrate the presence of chlorinated degradation intermediates that are only produced during biological reductive dechlorination (Table 2) and genetic analysis demonstrated the presence of *Dehalococcoides*, so dechlorinating bacteria are definitely present and active at the site. However, bacteria able to reductively dechlorinate TCE are slow growing. It is apparent that the degradation rate of TCE is significantly slower than that of vinyl chloride (Figure 5) and that an incubation period longer than 184 days is required to demonstrate degradation in these microcosms.

Analysis of sediment carbon as a source of secondary carbon for biodegradation

All of the field data indicate that there is active biological degradation occurring at the Building 51/64 Site (Tables 1 and 2). A source of organic carbon is necessary to maintain biodegradation activity against chlorinated solvents, so tests were conducted to determine if there was sufficient organic matter available at the site to support bacteria activity, particularly reductive dechlorination, for an extended period. Total sediment organic carbon was measured

as well as two indicators of available organic carbon, soluble organic carbon in the groundwater and readily biodegradable sediment carbon. The total organic carbon content of the sediment was between 2% to 5% of its dry weight (Table 5), indicating the presence of a potential carbon source to drive the reductive dehalogenation of chlorinated solvents.

Not all sediment carbon is readily available to bacteria, for example, soluble organic carbon is typically more bioavailable than insoluble, particulate organic carbon. Significant concentrations of soluble organic carbon (TOC) were present in site wells (Table 5), indicating that the naturally occurring sediment organic carbon is being dissolved into the groundwater. This suggests that the sediment organic matter will be bioavailable for chlorinated solvent degradation.

To firmly establish whether the sediment organic carbon is bioavailable, we measured the aerobic first-order biodegradation rate for the sediment organic carbon and measured the 30-day oxygen demand of the sediment (Table 5). All of the sediment samples from both the saturated and unsaturated zones had high concentrations of biologically available carbon, as indicated by 30-day oxygen demand measurements (Table 5). The first-order degradation rates were high enough (Table 5) to suggest that carbon availability is unlikely to be the rate-limiting step in chlorinated compound degradation at this site. The amount of sediment carbon present (over 2% of the sediment dry weight, Table 5) suggests that there is enough bioavailable sediment carbon to support chlorinated compound degradation for an extended period.

Summary and Conclusion

In summary, the results of this study are supportive of applying MNA for remediation of the Building 51/64 Site. TEA and methane data indicate an active microbial population at the site, chlorinated compound measurements demonstrate the presence of compounds that are well known microbial degradation products of the chlorinated solvents contaminating the site, biological analysis indicates that environmental conditions favorable for MNA exist at the site, bacterial studies show that chlorinated solvent degrading bacteria are present at the site, including *Dehalococcoides*, which can completely mineralize chlorinated solvents under anaerobic conditions, and sediment and groundwater organic carbon analysis shows that there is a substantial pool of bioavailable organic carbon present to drive forward reductive dechlorination and other degradative processes. In total, the results of this study confirm and further support the results of previous investigations (Conrad 2000, SCFA 2000) that recommended the application of MNA at this site.

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Table 1. Summary of terminal electron acceptor (TEA) and methane concentration data by biological zone.

Biological Zone	Water Level[1] (feet above mean sea level)	Dissolved Oxygen[2] (mg/L)	Ferrous Iron[2] (Fe^{+2} mg/L)	Divalent Manganese[2] (Mn^{+2} mg/L)	Nitrate[2] (NO_3-N mg/L)	Sulfate[2] (SO_4 mg/L)	Methane[3] (CH_4 µg/L)
Zone 1							
Mean	730	6.6	0.00	0.00	2.40	51.0	NA
SD	14						
n	4	1	1	1	1	1	
Zone 2							
Mean	700	1.5	0.05	0.00	2.10	47.5	0.0
SD	2.8	1.4	0.06	0.00	1.83	33.5	0.0
n	2	4	4	4	4	4	2
Zone 3							
Mean	691	2.0	0.13	1.23	0.77	26.3	2.5
SD	3.6	1.6	0.15	1.96	0.83	11.4	3.5
n	3	3	3	3	3	3	2
Zone 4							
Mean	681	0.7	3.75	11.20	1.70	17.0	2171
SD	6.9	0.1	2.62	9.62	2.26	9.9	2173
n	4	2	2	2	2	2	5
Zone 5							
Mean	627	0.3	3.75	8.05	1.70	49.0	NA
SD	37	0.2	5.16	5.59	0.70	8.5	
n	3	2	2	2	2	2	

[1]Fourth quarter 1999; [2]December 1997; [3]October 2002

Table 2. Summary of chlorinated organic compound concentration historical data[1] by biological zone.

Biological Zone	TCE (µg/L)	PCE (µg/L)	1,1,1-TCA (µg/L)	1,1-DCA (µg/L)	1,1-DCE (µg/L)	cis-1,2-DCE (µg/L)	Vinyl chloride (µg/L)	Total VOC (µg/L)
Zone 1								
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n	4	4	4	4	4	4	4	4
Zone 2								
Mean	22.4	1.2	0.8	2.1	1.0	4.3	0.0	32.2
SD	28.5	1.3	1.4	3.7	1.7	6.7	0.0	41.0
n	3	3	3	3	3	3	3	3
Zone 3								
Mean	43.0	5.8	0.0	8.0	4.2	115.7	14.0	201.0
SD	68.5	8.0	0.0	13.0	4.7	199.4	24.2	335.2
n	3.0	3	3	3	3	3	3	3
Zone 4								
Mean	1.9	0.6	0.0	4.1	3.8	6.6	9.9	26.5
SD	1.0	0.7	0.0	3.7	3.8	9.8	12.8	17.4
n	3.0	4	4	4	4	4	4	4
Zone 5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SD	3	3	3	3	3	3	3	3
n								

[1] Using mean data (n = 4) for fiscal year 1999 for wells in each zone.

Table 3. Screen intervals and depth to groundwater for wells adjacent to soil borings taken January 13, 2003

Well No.	Zone	Screen Interval (feet bgs[1])	Minimum depth to groundwater (feet bgs)	Maximum depth to groundwater (feet bgs)
51-96-16	3	10-30	17.2	19.5
51-97-13	4	48-68	30.5	36
56-98-2	4	35-55	13.7	23.7
51-97-12	4	29.5-49.5	30.8	36.4
51-97-15	5	88-108	70.6	72.1

[1]bsg, below ground surface

Table 4. Sediments collected from Building 51/64 contained substantial populations of aerobic and anaerobic heterotrophic bacteria, including populations of toluene degrading bacteria that contain chlorinated solvent degrading enzymes.

Sediment Samples	SB51-03-2 Seasonal	SB51-03-2 Saturated	SB51-03-1 Seasonal	SB51-03-1 Saturated	SB51-97-3 Seasonal	SB51-97-3 Saturated	SB56-98-03 Seasonal	SB56-98-03 Saturated
Associated Well	51-96-16	51-96-16	51-97-12	51-97-12	51-97-13	51-97-13	56-98-2	56-98-2
Zone	3	3	4	4	4	4	4	4
Processed Core Interval (feet)	18'-20'	23'-25' 26'-28'	32'-34' 34'-36'	42'-44' 44'-46'	32'-34' 34'-36'	40'-42' 42'-44'	19'-21' 21'-23'	27'-29' 31'-33'
Aerobic Heterotrophic Plate Count (CFU/g dry sediment)	$1.2x10^6$	$2.0x10^5$	$8.3x10^5$	$2.2x10^5$	$1.6x10^5$	$2.9x10^5$	$1.8x10^4$	$8.3x10^4$
Anaerobic Heterotrophic Plate Count (CFU/g dry sediment)	na[1]	$5.0x10^4$	na	$7.0x10^3$	na	na	na	$2.0x10^4$
Toluene Degrading Bacteria Plate Count (CFU/g dry sediment)	$7.6x10^3$	$4.3x10^4$	$1.2x10^5$	$4.6x10^4$	$4.8x10^3$	$4.9x10^6$	$1.1x10^3$	$1.3x10^6$

[1]na, not analyzed.

Table 5. Sources of natural electron donor potential needed to maintain reductive dechlorination during monitored natural attenuation at Site 51/54.

	SB51-03-2 Seasonal	SB51-03-2 Saturated	SB51-03-1 Seasonal	SB51-03-1 Saturated	SB51-97-3 Seasonal	SB51-97-3 Saturated	SB56-98-03 Seasonal	SB56-98-03 Saturated
Associated Well	51-96-16	51-96-16	51-97-12	51-97-12	51-97-13	51-97-13	56-98-2	56-98-2
Zone	3	3	4	4	4	4	4	4
Soluble organic carbon in groundwater (mg/L)	4.68		5.96		2.29		2.28	
Total organic carbon in sediment (% of dry weight)	4.3	2.4	3.5	2.7	3.1	3.9	3.6	2.8
Bioavailable carbon (μg of O_2/g sediment organic carbon/day)	0.136	0.067	0.120	0.265	0.099	0.066	0.142	0.079
Sediment oxygen demand (μg of O_2/g sediment/30 day)	1086	788	1029	1070	951	971	989	877

Figure 1. Extent of groundwater contamination as shown by total halogenated hydrocarbons (µg/L) isopleths at Building 51/64 Site using data from September 2000 (from SCFA 2002).

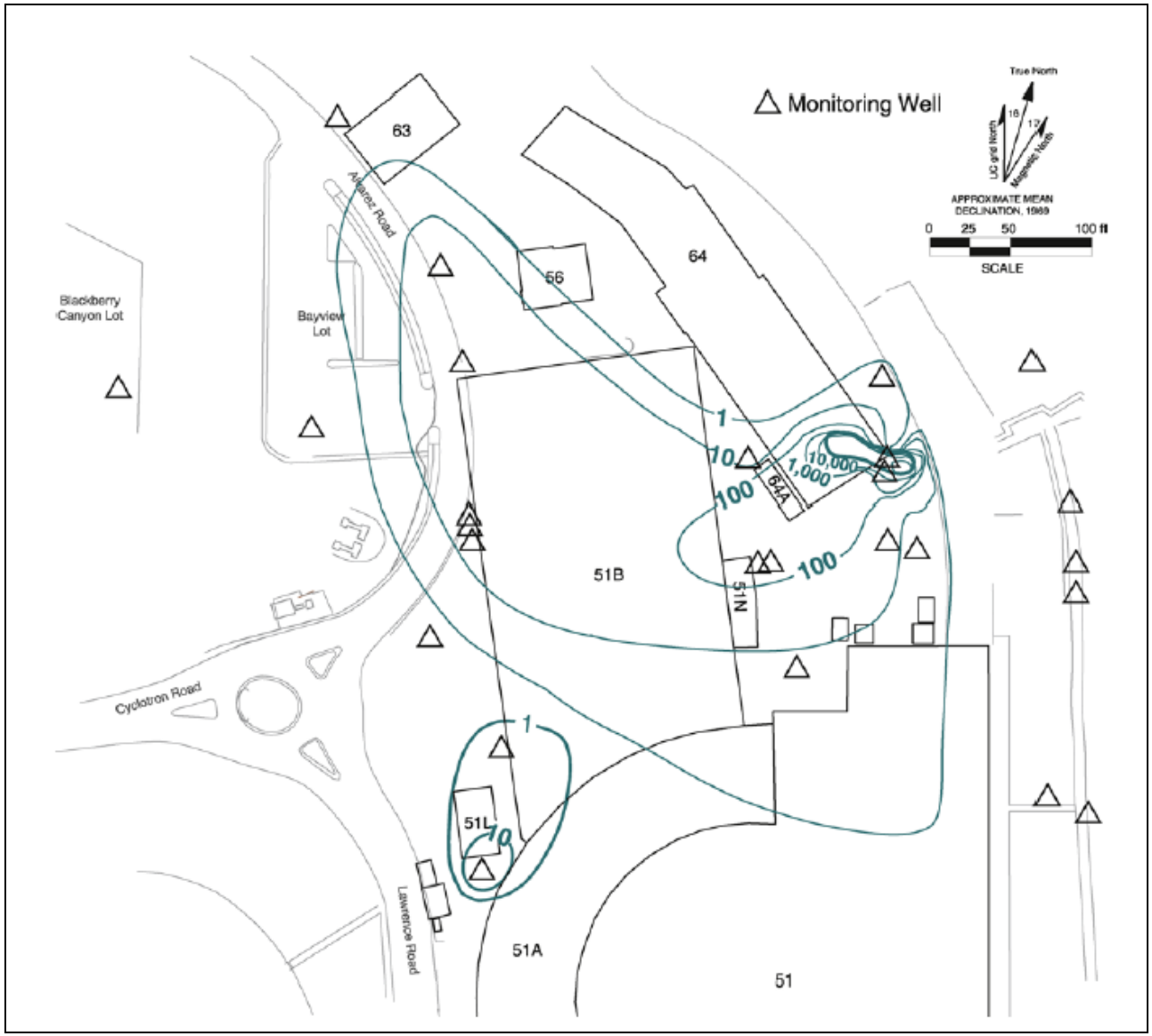


Figure 2. Competing Terminal Electron Acceptors and Chlorinated Solvent Degradation.

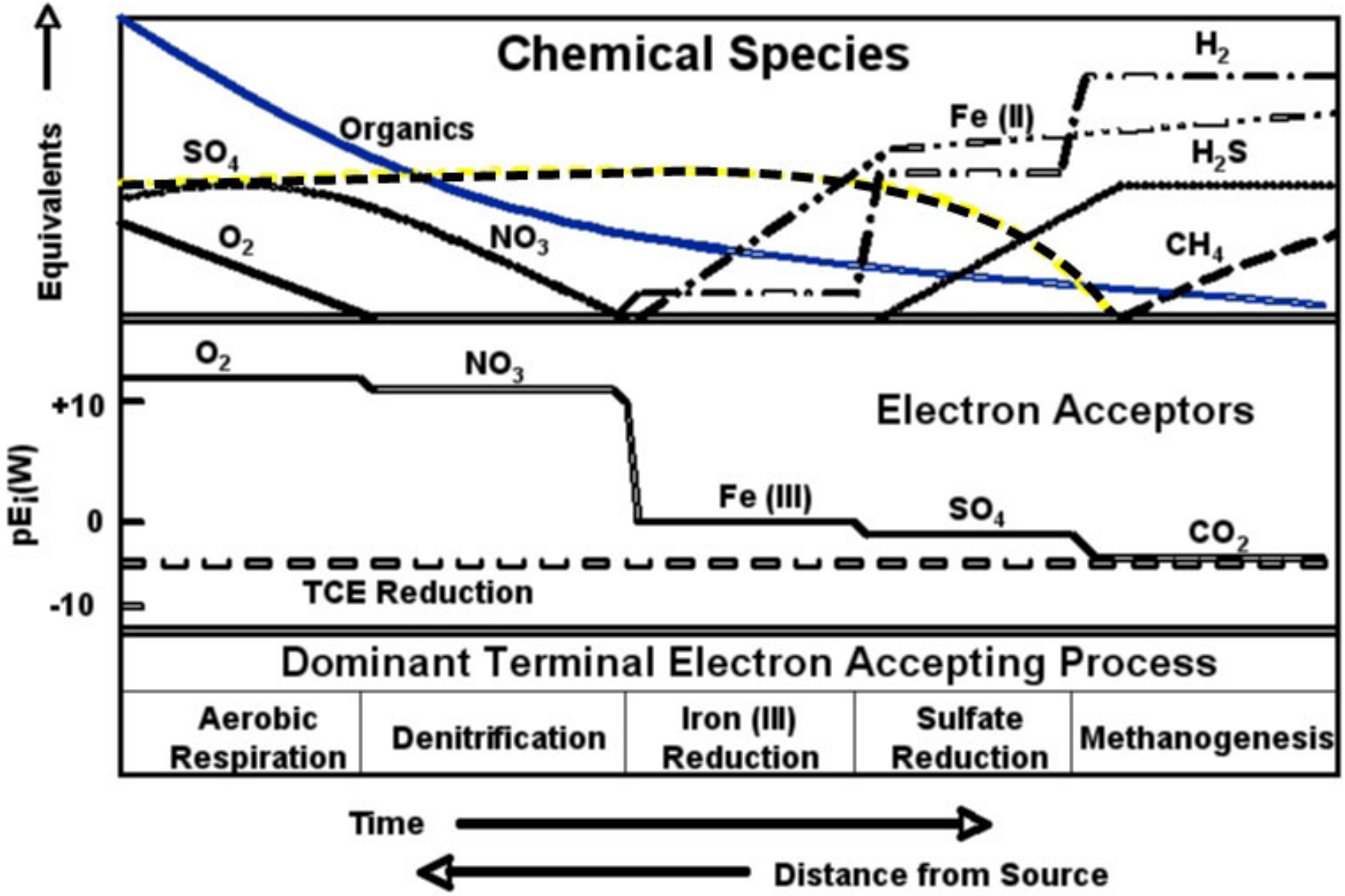


Figure 3. Map of biological zones and associated wells for the Building 51/64 Site.

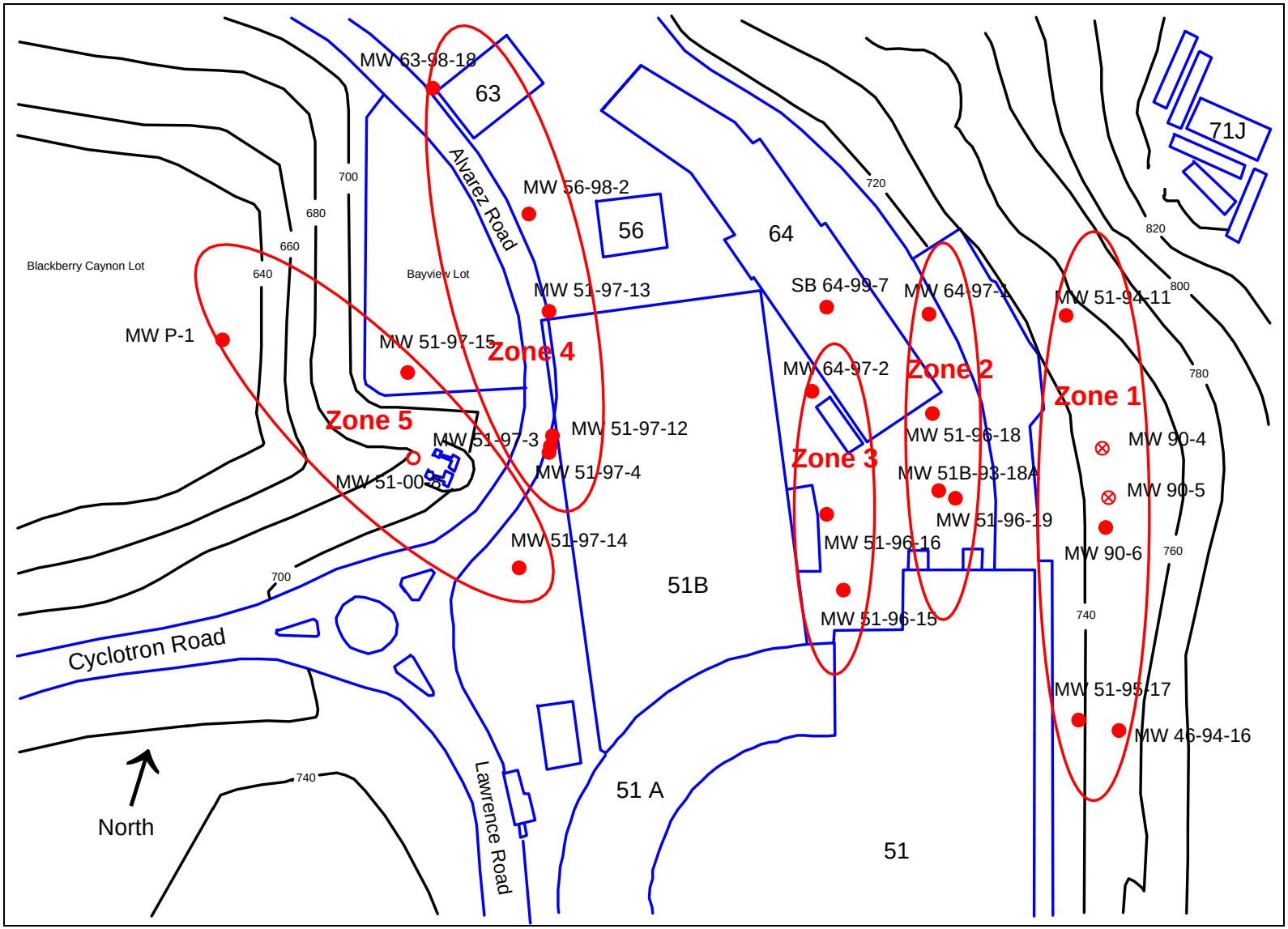
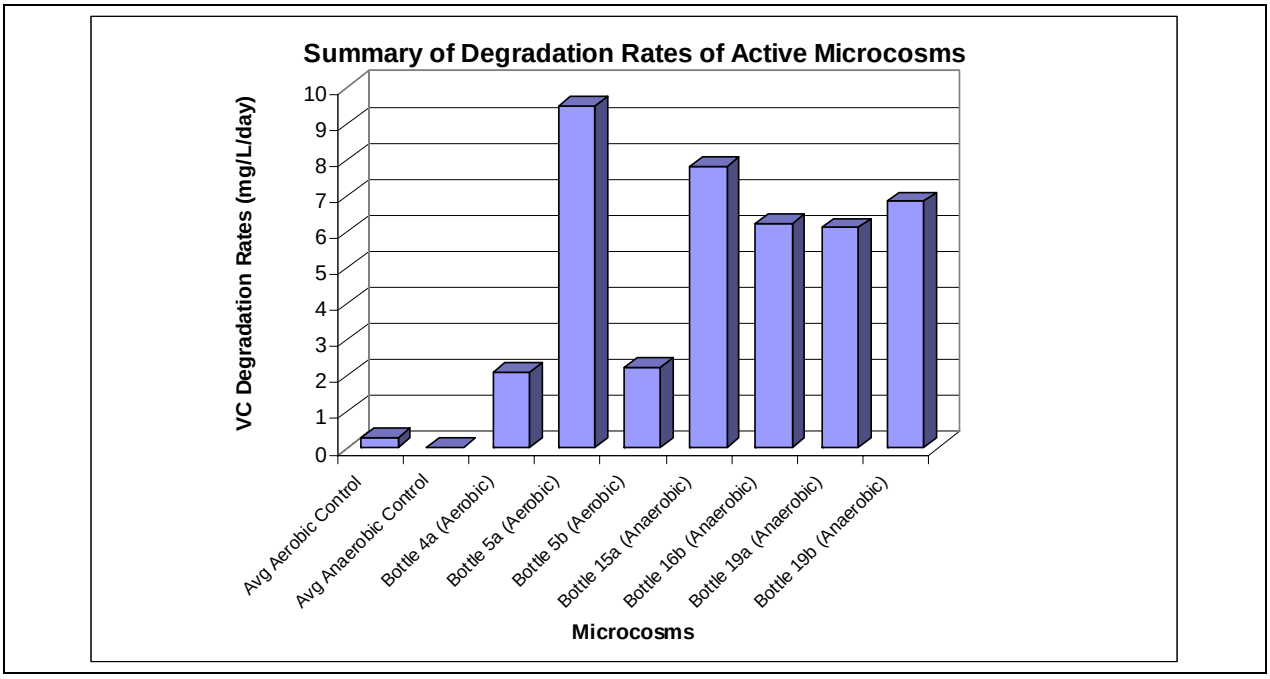


Figure 4. Comparison of maximum net degradation rates of active microcosms made from sediments collected at the Building 51/64 Site (from West et al. 2003).



VC, vinyl chloride

Figure 5. Typical graph of vinyl chloride biodegradation and ethene production observed in microcosms made from sediments collected at the Building 51/64 Site (from West et al. 2003).